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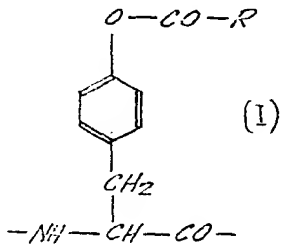
(54) PROCESS FOR THE MANUFACTURE OF TYROSINE-CONTAINING PEPTIDES AND THE DERIVATIVES THEREOF

(71) We, FARBWERKE HOECHST AKTIENGESELLSCHAFT, Vormals Meister Lucius & Bruning, of 6230 Frankfurt (M)-80, P.O. Box 800320, Federal Republic of Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention provides a process for the manufacture of tyrosine-containing peptides, wherein a peptide containing at least one tyrosine unit of the general formula



(I)

in which R represents an alkoxy radical derived from a primary or secondary alcohol, an aralkoxy radical containing at least 2 carbon atoms between the phenyl nucleus and the oxygen atom, or an NHR_1-group, in which R_1 represents a hydrogen atom or an alkyl, aralkyl or aryl radical, is

a) treated with ammonia, an amine, a hydrazine or, when R is not an NHR_1 group, with a mono - acyl - hydrazine, the amine or hydrazine derivative containing at least one NH-group; or

b) subjected to alkaline hydrolysis; or

c) treated with an alkali metal alcoholate; or

d) treated with a solution of an alkali or alkaline earth metal in liquid ammonia.

For the synthesis of tyrosine-containing peptides, the hydroxyl group of the tyrosine often remains unprotected or it is converted into a benzyl ether, a tertiary butyl ether or into the benzyloxy - carbonyl compound (cf. R. Schroder and K. Lubke, "The peptides", New York and London, Volume I, (1965) pages 220—226). If the OH-group is not protected, side-reactions often occur. In the synthesis of higher peptides using the above-mentioned protective groups, the sensitivity to acid or to catalytically activated hydrogen has often a disturbing effect on the benzyl derivatives.

The O-protective groups of the tyrosine used according to the process of the present invention do not have these disadvantages, because they cannot be split off either in an acid medium or by hydrogenation.

Thus, the use of the new O-protecting group permits the stepwise building up of tyrosine-containing peptides from the carboxyl end while retaining the O-protective group. With the hitherto used protective groups this was often very difficult because the separation of the N-protective group often also entailed separation of the O-protective group.

The substituted tyrosine derivatives, in which R represents an alkoxy or aralkoxy group as defined above and which are re-

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quired for the preparation of the protected tyrosine peptides used in the process of the present invention, may be obtained by the reaction of N - acyl - tyrosine with a chloroformic acid alkyl ester in the presence of an acid-binding agent.

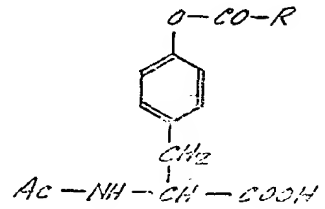
In the alkoxycarbonyl - tyrosine, the alkyl group of the alkoxy group R in the general formula (I) may be, more especially, a lower aliphatic alkyl radical, for example, a methyl, ethyl, n - propyl, isopropyl or isobutyl group or a cycloaliphatic group such, for example, as a cyclohexyl group. As the aralkyl residue of the aralkoxy groups, there may be used, for example, β - phenyl- ethyl, γ - phenyl - propyl, 4 - phenyl - cyclohexyl or dibenzyl - methyl group.

Tyrosine derivatives, in which R stands for the group NHR_1, and which are required for the preparation of the protected tyrosine peptides used according to the present invention, may be obtained by methods known per se, by the reaction of an N - acyl - tyrosine ester with an N - carbonyl - sulphamic acid chloride [cf. German Patent 931,225, German Patent 931,467, Chemische Berichte 89, 1071 (1956), Chemische Berichte 96, 56 (1963)] with urea chloride [cr. Liebigs Annalen 244, 29 (1888)] or with an alkyl- or aryl - isocyanate.

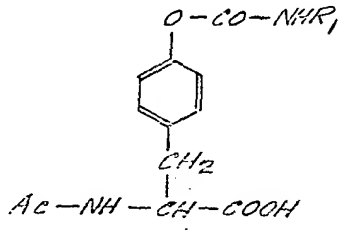
As alkyl groups R_1 of the group NHR_1 in the carbamyl compounds ($R=NHR_1$), there may be used, more especially, lower aliphatic alkyl groups, for example, a methyl, ethyl, n - propyl, isopropyl or isobutyl groups or cycloaliphatic groups such, for example, as a cyclohexyl group. As aralkyl groups of the aralkyl - amido group, there may be used, for example, a β - phenyl - ethyl, γ - phenyl - propyl, 4 - phenyl - cyclohexyl or a dibenzylmethyl group. As aryl groups R, there may be mentioned, more especially, a phenyl group and substituted phenyl groups as well as a naphthyl group.

The new protective groups may also be introduced into N - acyl - tyrosine esters and into tyrosine - containing peptides, if no other acylatable groups are present.

Preferred tyrosine derivatives which may be used in the preparation of tyrosine-containing peptides of the general formula I are those of the general formula



wherein Ac represents a carbobenzoxy, tertiary butyloxy - carbonyl or a formyl radical and R represents an alkoxy radical containing from 1 to 4 carbon atoms, and those of the general formula



wherein Ac represents a carbobenzoxy or a tertiary butyloxy - carbonyl radical and R_1 represents a hydrogen atom or an isobutyl, phenyl or 4 - nitro - phenyl radical.

These compounds may now be used according to the general methods of peptide chemistry for the manufacture of tyrosine-containing peptides. By direct reaction of the N - acyl - O - alkyloxy - carbonyl - tyrosine with, for example, an amino - acid ester or peptide ester or peptide - amine in the presence of a condensing agent such, for example, as dicyclohexyl - carbodiimide (DCC), there is formed a peptide with prolongation of the chain at the carboxyl end of the tyrosine. By setting free the carboxyl group, for example, by catalytic hydrogenation of N - tertiary butyloxy - carbonyl - O - phenylcarbamyl - tyrosine - benzyl ester and subsequent condensation of the N - tertiary butyloxy - carbonyl - O - phenyl - carbamyl - tyrosine so formed with an amino - acid ester or a peptide ester, there is likewise obtained a peptide with extension of the chain at the carbonyl end of the tyrosine.

By splitting off the N - acyl group, for example by catalytic hydrogenation of N - carbobenzoxy - O - ethyloxy - carbonyl - tyrosine methyl ester, there is formed the O - ethyloxycarbonyl - tyrosine methyl ester which can be reacted as well as an O - carbamyl - tyrosine ester obtained upon separation of the N - acyl group, with an N - acyl - amino acid or -peptide to yield a new peptide with prolongation of the chain at the amino end of the tyrosine.

It is also possible to use other condensation methods of peptide chemistry, for example, the mixed anhydride method or the peptide synthesis via active ester. Active esters are accessible, for example, from N - acyl - O - alkyl - oxycarbonyl - tyrosine and the activating component, in most cases 4 - nitro phenyl, 2,4,5 - trichlorophenol, pentachloro - phenol or N - hydroxy - succinimide, in the presence of dicyclohexyl - carbodiimide.

A further units of the peptides containing the tyrosine derivatives of the general formula (I), there may be used all amino - acids in

their L- or D-form present in naturally occurring peptides. Even the use of β - amino - acids, for example, β - alanine, or of other, only synthetically or semi - synthetically accessible amino - acids, for example α - methyl - alanine, α - methyl - 3,4 - di - hydroxy - L - phenylalanine or β - cholor - alanine, is possible. Further functional groups of the amino - acids are suitably protected according to the method commonly used in peptide chemistry (cf. E. Schroder and K. Lubke, "The Peptides", New York and London 1965, Volume I, especially pages 3—75).

The separation of the new O - protective groups succeeds according to the invention by treatment with nucleophilic reagents such as hydrazine and the alkyl, aryl and mono - acyl derivatives thereof which still contain at least one -NH group, for example, methyl - hydrazine, phenyl - hydrazine, methyl- or tertiary - butyloxycarbonyl - hydrazide, furthermore with ammonia, primary and secondary, preponderantly lower aliphatic amines, for example, methyl - amine, ethyl-amine, propylamine, isopropylamine, butyl - amine, isobutylamine, dimethylamine or di - butylamine. The separation is also possible with the salts of hydrazines or amines or of ammonia with weak organic acids, preferably acetic acid. Mono - acyl - hydrazines, however, are not suitable for the separation of the O - carbamyl - protective groups (R= NHR_1), because the carbamyl - protective groups are more difficult to separate than the carbalkoxy - protective groups.

The alkaline hydrolysis may be effected with aqueous or alcoholic alkali metal lye or alkaline earth metal lye, for example, sodium hydroxide solution, aqueous - methanolic barium hydroxide or lithium hydroxide, and with alkali metal alcoholates, for example, sodium methylate in methanol. Even when the peptide is treated with an alkali metal, for example, sodium, potassium or calcium, in liquid ammonia, the new protective groups are split off simultaneously.

The splitting conditions depend on the reagent used and on the nature of the peptide. With hydrazine, the separation of the protective groups will normally be completed in the case of alkoxycarbonyl-compounds after 30 minutes, and in the case of the carbamyl compounds mostly after a few hours, whereas ammonia and amines require longer reaction periods.

The alkoxycarbonyl - protective group is split off with 1N - sodium hydroxide solution after 30—60 minutes, the carbamyl group requires a longer reaction period of about 1—2 hours even with 2N - sodium hydroxide solution. The unsubstituted carbamyl-protective group is the most rapid to be removed.

Heating is in general not necessary, unless difficult solubility of the peptides in cold solvent makes it necessary.

As solvents, all solvents commonly employed in peptide chemistry may be used if they are stable towards the splitting reagents under the reaction conditions; thus, for example, water, alcohols, dioxane, dimethylacetamide and pyridine, or a mixture of any two or more of such solvents, may be used.

The isolation of the peptides resulting from the separation of the protective groups may be effected. if these peptides are soluble in, for example, ethyl acetate, by distribution between ethyl acetate and weakly acidified water. If they are insoluble in solvents such as ethyl acetate, acetone or ether, the peptides are simply precipitated with the aid of one of these solvents, preferably acetone, alone or in admixture with ethyl acetate or ether, from its solution. It is of advantage previously to remove by distillation a part of the solvent in which the separation has ben effected.

The products of the present invention may be used as medicaments or they may be used as intermediate products in the manumanufacture of other therapeutically valuable peptides.

Thus, for example For - Tyr - (ETOC) - Ser - Met - OMe (Example 3b) may be reacted, after separation of the protective groups, with BOC - Ser - N_2 to yield BOC - Ser - Tyr - Ser - Met - OH. From this compound, there is obtained by condensation with H - Glu (OtBu) - His - Phe - Arg - Trp - Gly - OH the MSH - active decapeptide of the ACTH - sequence 1—10. This can again be reacted according to German Patent 1,240,088 to yield the $ACTH^{1-39}$ - amide which, after separation of the protective groups and purification, has full ACTH-activity.

Furthermore, for example, the known heptapeptide H - Ile - Glu(NH_2) - Asp - (NH_2) - Cys - (Bzl) - pro - Leu - Gly - NH_2 from the oxytocin sequence may be reacted with Z - Tyr(EtOC) - ONP, BOC - Tyr(PAC) - OTCP or with another activated tyrosine derivative of this series to yield the corresponding octapeptide derivative and this may may further reacted, after separation of the N-protective group, with BOC - Cys(Bzl) - ONP to yield the protected oxytocin derivative. The separation of the Bzl - protective groups with sodium in liquid ammonia, whereby according to the present invention the new protective groups are simultaneously removed, and the further reaction steps towards oxytocin are known.

By similar methods, known per se, the new tyrosine derivatives may be used for the synthesis of other peptides, for example insuline, glucagon and hypertensin.

The invention also provides a pharmaceutical preparation comprising a tyrosine-

containing peptide obtained in accordance with the invention, in admixture or conjunction with a pharmaceutically suitable carrier.

The following Examples illustrate the invention. The abbreviations used for denoting the individual amino - acids and protective groups are those commonly used in peptide chemistry:

Z=carbobenzoxy
For=formyl
ONP=p - nitrophenyl
BOC=tertiary - butyloxycarbonyl
TCP=2,4,5 - trichlorophenyl
Bzl=benzyl

For the new O-protective groups of the tyrosine, the following abbreviations are introduced:

EtOC=ethyl - oxycarbonyl
AC=carbamyl
i - BAC=iso - butyl - carbamyl
PAC=phenyl - carbamyl
NPAC=nitrophenyl - carbamyl

Example 1

a) Z - Tyr - (EtOC) - OH

31.5 g (0.1 mol) of Z - Tyr - OH were dissolved in 150 ml of 1N - NaOH. The solution as combined with 15 g of sodium carbonate; then, 11 ml (0.115 mol) of chloroformic acid ethyl ester were added dropwise, while stirring vigorously, at 10°C at the most. After a short time, a thick precipitate was formed. The whole was diluted with 300 ml of water and stirred for one hour at room temperature. The pH was then adjusted to 2 by means of semi-concentrated HCl and the precipitate that had separated was taken up in 300 ml of ethyl acetate. The ethyl acetate solution was washed with 1N-HCl and water and dried over sodium sulphate. After removal by distillation of the ethyl acetate, a crystalline residue remained behind which was recrystallized from 150 ml of 60% methanol. Yield: 36.1 g (93% of the theoretical yield. Melting point 117—119°C.

$C_{20}H_{21}NO_7$ (387.37) Calc.:
C=62.1 H=5.47 N=3.61
Found:
C=61.7 H=5.5 N=3.9

In a manner analogous to that described in Example 1a), there were prepared:

N - carbobenzoxy - O - methyl - oxy - carbonyl - L - tyrosine, melting point 120—122°C;
N - carbobenzoxy - O - isobutyl - oxy - carbonyl - L - tyrosine, melting point 103—105°C;
N - carbobenzoxy - O - isopropyl - oxy - carbonyl - L - tyrosine, melting point 119—121.5°C;
N - tert.butyl - O - ethyl - oxycarbonyl - L - tyrosine, melting point 165—166° C.

b) Z - Tyr - (EtOC) - ONP

7.74 g (20 mmols) of Z - Tyr - (EtOC) - OH and 3.34 g (24 mmols) of 4 - nitro - phenol were dissolved in a mixture of 70 ml of ethyl acetate and 30 ml of dimethylformamide and combined, at 0°C, with 4.2 g (20.4 mmols) of dicyclohexyl - carbodiimide. After having allowed the whole to stand for 15 hours at 5°C, it was cooled to 0°C and the urea that had formed was filtered off with suction; the filtrate was evaporated to dryness under reduced pressure. An oily residue remained which crystallized upon rubbing with isopropanol. The yield, after three recrystallizations from isopropanol, amounted to 6.28 g (62% of the theory). Melting point: 111—112°C.

$C_{26}H_{24}N_2O_8$ (508.5) Calc.:
C=61.41 H=4.76 N=5.51
Found:
C=61.5 H=4.7 N=6.2

c) Z - Tyr - (EtOC) - Phe - OCH_3

1.08 g (5 mmols) of H - Phe - OCH_3.HCl and 2.54 g (5 mmols) of Z - Tyr - (EtOC) - ONP were dissolved in 15 ml of dimethylformamide and, after cooling to −5°C, combined with 0.69 ml (5 mmols) of triethyl - amine. After having allowed the whole to stand for 60 hours at room temperature, it was evaporated under reduced pressure, the solid residue was dissolved in ethyl acetate and washed 15 times with saturated sodium bicarbonate solution and one time each with 1N-HCl and water. The solution was dried over sodium sulphate and evaporated under reduced pressure. The residue was triturated with ether and after standing for some time in ether it was filtered with suction and washed with ether. Yield: 2.40 g (87.6% of the theory). Melting point: 176—176.5°C.

$C_{30}H_{32}N_2O_8$ (548.60) Calc.:
C=65.68 H=5.88 N=5.11
Found:
C=65.7 H=5.9 N=5.2

The compound was also prepared by acylation of the tyrosine peptide:

4.76 g (10 mmols) of Z - Tyr - Phe - OCH_3, prepared according to J. Amer. chem. Soc. 83 (1961), page 723, were dissolved in 25 ml of tetrahydrofurane and 60 ml of chloroform; after addition of 1.67 ml (12 mmols) of triethylamine, 1.09 ml (12 mmols) of chloroformic acid ethyl ester was added drop-

wise, while stirring, at 0°C. The solution was allowed to stand for 12 hours at room temperature. The solvent was then removed by distillation under reduced pressure and the residue was taken up in moist ethyl acetate. The ethyl acetate solution was washed consecutively with 2N-HCl, 1N-NaOH and water, dried over sodium sulphate and evaporated to dryness under reduced pressure. The residue was recrystallized from a mixture of methanol and water. Yield: 3.6 g (66% of the theory). Melting point 175—175.5°C.

d) Z - Tyr - Phe - N_2H_3

1.37 g 2.5 mmols) of Z-Tyr-(EtOC)-Phe-OCH_3 was dissolved in 100 ml of methanol and allowed to stand for 70 hours at room temperature with 0.95 ml (15 mmols) of 80% hydrazine hydrate. The crystalline precipitate was filtered off with suction and washed with methanol. Yield: 0.97 g (81.5% of the theory). For analysis, it was recrystallized from 500 ml of 80% methanol. Yield: 0.79 g (66.5% of the theory). Melting point 241.5°C.

$C_{25}H_{28}N_4O_5$ (476.54) Calc.:
C=65.53 H=5.92 N=11.76
Found:
C=65.9 H=6.0 N=11.6

Example 2

a) Z - Tyr - (EtOC) - OCH_3

6.60 g (20 mmols) of Z - Tyr - OCH_3 were dissolved in 50 ml of chloroform. After addition of 3.22 ml (23 mmols) of triethylamine, 2.17 ml (23 mmols) of chloroformic acid ethyl ester were added dropwise, while stirring, at 0°C. After having allowed the mixture to stand for 3 hours at room temperature, it was washed each time twice with saturated sodium bicarbonate solution, 1N-HCl and water, dried over sodium sulphate and evaporated. Yield: 7.2 g (90% of the theory); melting point 94—95°C. After recrystallization from diisopropyl ether, 6.5 g (81% of the theory) of substance were obtained. Melting point 95—95.5°C.

$C_{21}H_{23}NO_7$ (401.40) Calc.:
C=62.83 H=5.78 N=3.49
Found:
C=62.7 H=5.7 N=3.5

b) H - Tyr - (EtOC) - OCH_3.HCl

4.01 g (10 mmols) of Z - Tyr - (EtOC) - OCH_3 were dissolved in 100 ml of methanol and, after addition of 2.09 ml of 4.97N-HCl (10.3 mmols) hydrogenated for 2 hours in the presence of palladium black. After removal of the catalyst by filtration, the filtrate was evaporated to dryness under reduced pressure and the crystal mass that remained was triturated with ether and hot ethyl acetate and filtered with suction. Yield: 1.9 g (62% of the theory). Melting point 162—163°C.

$C_{13}H_{18}ClNO_5$ (303.75) Calc.:
C=51.40 H=5.97 N=4.61 Cl=11.6
Found:
C=51.8 H=6.0 N=4.9 Cl=12.0

c) Z - Phe - Tyr - (EtOC) - OCH_3

1.49 g (5 mmols) of Z - Phe - OH were dissolved in 15 ml of tetrahydrofurane. After addition of 0.7 ml (5 mmols) of triethylamine, 0.48 ml (5 mmols) of chloroformic acid ethyl ester was added dropwise, while stirring, at −10°C. The whole was stirred for 5 minutes at −5°C and combined with the solution of 1.52 g (5 mmols) of H - Tyr.-(EtOC) - CH_3.HCl and 0.7 ml of triethylamine in 20 ml of dimethyl - acetamide, which had been previously cooled to −5°C. The temperature of the batch was allowed to rise slowly to room temperature and then the mixture was stirred for 2 hours at this temperature. The solvents were then removed by distillation under reduced pressure, the residue was taken up in ethyl acetate and the ethyl acetate solution was washed each time thrice with saturated sodium bicarbonate solution, 1N-HCl and water, dried over sodium sulphate and evaporated under reduced pressure. 2.3 g (84% of the theory) of substance were obtained. Melting point 163—166°C. After recrystallization from a mixture of acetone and water, 1.88 g (69% of the theory) of substance were obtained. Melting point: 170.5—171.5°C.

$C_{30}H_{32}N_2O_8$ (548.60) Calc.:
C=65.68 H=5.88 N=5.11
Found:
C=65.7 H=5.9 N=5.2

d) Z - Phe - Tyr - OH

2.75 g (5 mmols) of Z - Phe - Tyr - (EtOC) - OCH_3 were dissolved in 40 ml of dioxane and stirred for 2 hours at room temperature with 15 ml of 1N-NaOH. The whole was diluted with 250 ml of water and by adding 15 ml of 1N-HCl a semi-solid precipitate was separated which was dissolved in ethyl acetate and extracted from the ethyl acetate solution by means of a sodium bicarbonate solution. Upon addition of 1N-HCl, a crystalline precipitate separated which was dried under reduced pressure over P_2O_5. The compound was found to melt at 181.5—183° C and was identical with an authentic sample prepared according to Liebigs Annalen der Chemie, 652 (1962), page 76.

Example 3

a) For - Tyr - (EtOC) - OH

20.9 g of For - Tyr - OH (0.1 mol) were dissolved in 150 ml of 1N-NaOH. 15 g of sodium carbonate were added and then 11.0

ml (0.115 mol) of chloroformic acid ethyl ester were added dropwise, while stirring vigorously, at 10°C at the most. The whole was stirred for 2 hours at room temperature and by adding 2N-HCl a white crystalline precipitate was separated. The crystals were dissolved in ethyl acetate, the filtrate was extracted twice with ethyl acetate, the combined ethyl acetate solutions were washed with 1N-HCl and water, dried over sodium sulphate and evaporated under reduced pressure. A crystalline residue remained which was recrystallized from 25% methanol. Yield: 23.0 g (81.5% of the theory). Melting point 172—173°C.

$C_{13}H_{15}NO_6$ (281.26) Calc.:
C=55.55 H=5.38 N=4.94
Found:
C=55.6 H=5.3 N=5.1

b) For - Tyr - (EtOC) - Ser - Met - OCH_3

7.0 g (20 mmols) of BOC - Ser - Met - OCH_3, prepared according to German Specification 1,212,981 laid open to public inspection, were dissolved in 54 ml of 0.55 N-HCl in methanol. The solution was allowed to stand for one hour at room temperature, the solvent was removed by distillation under reduced pressure and the oily residue was digested several times with anhydrous ether. The excess ether was removed under reduced pressure. The residue was dissolved in 40 ml of a mixture of dimethyl - acetamide and acetonitrile 1:1. 5.62 g of For - Tyr - (EtOC) - OH (20 mmols) and 2.81 ml (20 mmols) of triethylamine were added and the whole was combined at −15°C with 4.3 g (21 mmols) of DCC dissolved in a small amount of acetonitrile. The temperature of the mixture was then allowed to rise slowly to room temperature and, after standing overnight, the urea that had precipitated (4.5 g) was removed by filtration with suction. The filtrate was evaporated to dryness under reduced pressure, the residue was taken up in moist ethyl acetate, the ethyl acetate solution was washed, after filtration, with 1N-HCl, saturated sodium bicarbonate solution and water (the aqueous phase each time containing 10% of NaCl) and evaporated to dryness with addition of toluene. The residue was recrystallized from ethyl acetate, during which operation a small amount of undissolved matter was removed by filtration. Yield: 7.6 g (74% of the theory). Melting Point: 164—166°C.

$C_{22}H_{31}N_3O_6S$ (513.56) Calc.:
C=51.4 H=6.09 N=8.18
Found:
C=51.3 H=6.3 N=8.3

c) For - Tyr - Ser - Met - N_2H_3

1.54 g (3 mmols) of For - Tyr - (EtOC) - Ser - Met - OCH_3 were dissolved in 20 ml of methanol. 1.1 ml (18 mmols) of 80% hydrazine hydrate was added and the whole was allowed to stand for 12 hours at room temperature. The precipitate (1.22 g) was separated by filtration with suction and recrystallized from 60 ml of 80% methanol. Yield: 0.98 g (74% of the theory). Melting point: 208—210°C.

$C_{18}H_{27}N_5O_6S$ (441.50) Calc.:
C=48.95 H=6.16 N=15.82
Found:
C=48.6 H=6.3 N=16.0

EXAMPLE 4

a) Z - Tyr - (EtOC) - Gly - NH_2

7.7 g of Z - Tyr - (EtOC) - OH (20 mmols) and 2.8 ml of triethylamine were dissolved in 40 ml of tetrahydrofurane. 2.6 ml of chloroformic acid isobutyl ester were added dropwise, while stirring, at −5°C, and the whole was stirred for 10 minutes at −5°C and then combined with the solution of 3.73 g of H - Gly - NH_2.HCl (30 mmols) and 4.0 ml (28.8 mmols) of triethylamine in a small amount of water which had been cooled to 0°C. The whole was stirred for one hour at room temperature, the solvent was removed by distillation under reduced pressure, the residue was taken up in moist ethyl acetate and the ethyl acetate solution was washed with 1N-HCl, saturated sodium bicarbonate solution and water, the ethyl acetate was removed by evaporation and the residue was crystallized from 70% ethanol. 8.08 g (91% of the theory) of jelly-like crystals melting at 154—157°C (sintering at 150°C) were obtained. For analysis, the compound was recrystallized from diethyl - ketone. Melting point: 157—159°C.

$C_{22}H_{25}N_3O_7$ (443.44) Calc.:
C=59.6 H=5.68 N=9.48
Found:
C=59.4 H=5.6 N=9.5

b) Z - Tyr - Gly - NH_2

4.43 g (10 mmols) of Z - Tyr - (EtOC) - Gly - NH_2 were stored for 12 hours at room temperature in 40 ml of 2N-NH_3 in methanol. The whole was evaporated to dryness and the residue was recrystallized from a mixture of methanol and water. Yield: 3.42 g (92% of the theory). Melting point 114—116°C. The compound was identical with the amide prepared from the ethyl ester [Chem. Ber 93 (1960), page 2387].

EXAMPLE 5

Separation of the N - BOC protective group under maintenance of the new O-protective group:

50 ml of saturated HCl/ethyl acetate were poured over 3.53 g (10 mmols) of POC -

Tyr - (EtOC) - OH. After 30 minutes, the precipitate was separated by filtration with suction and washed with ethyl acetate and ether. Yield: 2.30 g (79.3% of the theory). Melting point: 219—220°C (decomposition).

$C_{12}H_{14}ClNO_5$ (289.72) Calc.:
C=49.75 H=5.57 N=4.84 Cl=12.24
Found:
C=49.9 H=5.6 N=5.5 Cl=12.3

By disolving the hydrochloride in 10 ml of hot water and addition of 1 ml of pyridine, the O - ethyloxycarbonyl - L - tyrosine could be separated and was then filtered off with suction and recrystallized from water.

EXAMPLE 6

a) Z - Tyr - (AC) - OCH_3

α) 3.29 g (10 mmols) of Z - Tyr - OCH_3 were dissolved in 4 ml of acetonitrile and combined, while cooling, with 0.89 ml (10 mmols) of N - carbonyl - sulphamic acid chloride. After having allowed the mixture to stand for 4 hours at room temperature, it was poured into 500 ml of water, heated for 15 minutes to 70°C and then cooled to 5°C. The crystals, which had been filtered off with suction, were washed with water until they were free from acid, dried and recrystallized twice from a mixture of chloroform and ether. Yield: 1.75 g (47% of the theory), melting point: 131.5—132°C.

$C_{21}H_{24}N_2O_6$ (372.39) Calc.:
C=61.28 H=5.41 N=7.52
Found:
C=61.2 H=5.5 N=7.4

β) 3.29 g (10 mmols) of Z - Tyr - OCH_3 were dissolved in 10 ml of methylene chloride, 0.87 g (11 mmols) of urea chloride was added, and the solution was allowed to stand for 4 hours at room temperature. After evaporation to dryness, the solid residue was triturated with water, filtered off with suction and washed with water until neutrality. The dried crude product was recrystallized thrice from a mixture of chloroform and ether. Yield: 1.82 g (49% of the theory). Melting point: 131.5°C.

b) H - Tyr - (AC) - OCH_3.HCl

1.86 g (5 mmols) of Z - Tyr - (AC) - OCH_3 were dissolved in 30 ml of methanol and, after addition of 0.8 ml (5.8 mmols) of 7.3N - methanolic HCl, hydrogenated for 2 hours in the presence of palladium black. The precipitate that had formed was dissolved by the addition of dimethylformamide and separated from the catalyst by filtration. The filtrate was freed from solvent under reduced pressure at room temperature. Yield: 1.25 g (91% of the theory); melting point: 214.5—215.5°C (decomposition).

$C_{13}H_{15}N_2O_5Cl$ (274.71) Calc.:
C=48.09 H=5.50 N=10.20 Cl=12.91
Found:
C=47.6 H=5.5 N=10.0 Cl=13.6

c) Z - Phe - Tyr - (AC) - OCH_3

The mixture of 0.82 g (3 mmols) of H - Tyr - (AC) - OCH_3.HCl and 1.43 g (3 mmols) of Z - Phe - OTCP in 50 ml of dimethylformamide was combined, at −5°C, with 0.43 (3 mmols) of triethylamine and stored for 65 hours at room temperature. The solution was then evaporated in the cold and in a high vacuum, the solid residue was dissolved in a large amount of chloroform and the solution was washed five times with sodium bicarbonate solution, twice with 1N - hydrochloric acid and twice with water. After drying over sodium sulphate, the solvent was removed by distillation under reduced pressure and the crystalline residue was triturated with ether, allowed to stand for some hours at 5°C and filtered off with suction. Yield: 1.03 g (66% of the theory). Melting point 187—188°C. For analysis, a sample was recrystallized from methanol. It showed no increase of the melting point.

$C_{29}H_{33}N_3O_7$ (519.46) Calc.:
C=64.73 H=5.63 N=8.09
Found:
C=64.5 H=5.7 N=8.3

d) Z - Phe - Tyr - $NHNH_2$

0.52 g (1 mmol) of Z - Phe - Tyr - (AC) - OCH_3 were dissolved in 4 ml of dimethyl - acetamide and allowed to stand for 18 hours, at room temperature, with 0.32 ml (5 mmols) of 80% hydrazine hydrate. The solid residue obtained upon evaporation of the solvent under reduced pressure at room temperature was triturated with methanol and filtered off with suction. The crude product melting at 210—212°C (0.37 g=78% of the theory) was recrystallized from methanol. Yield: 0.28 g (50% of the theory). Melting point: 224.5—225°C (decomposition).

$C_{25}H_{28}N_4O_5$ (476.54) Calc.:
C=65.53 H=5.92 N=11.76
Found:
C=65.2 H=6.0 N=12.4

EXAMPLE 7

a) Z - Tyr - (i - BAC) - OCH_3

3.29 g (10 mmols) of Z - Tyr - OCH_3 were dissolved in 10 ml of i - butyl - iso - cyanate and heated for 2 hours to 60°C. After cooling to 0°C, the whole was triturated successively thrice with ligroin and decanted. The crystals were washed with ligroin and recrystallized from a mixture of chloroform and ligroin. Yield: 3.28 g (76% of the theory). Melting point: 108.5°C.

$C_{25}H_{28}N_2O_6$ (428.49) Calc.:
C=64.47 H=6.59 N=6.54
Found:
C=64.2 H=6.6 N=6.7

b) H - Tyr - (i - BAC) - OCH_3.HBr

4.28 g (10 mmols) of Z - Tyr - (i - BAC) - OCH_3 were dissolved in 25 ml of a mixture of HBr and glacial acetic acid and allowed to stand for one hour at room temperature. After having poured the whole into 500 ml of absolute ether and stored for 30 minutes at 5°C, the crystals that had formed were separated by filtration with suction, triturated again in absolute ether, filtered off with suction and washed with ether. Yield: 3.28 g (87.5% of the theory). Melting point: 210.5—211.5°C (decomposition).

$C_{17}H_{21}N_2O_4Br$ (375.28) Calc.:
C=48.01 H=6.18 N=7.46 Br=21.29
Found:
C=48.1 H=6.0 N=7.0 Br=21.3

c) Z - Phe - Tyr - (i - BAC) - OCH_3

0.75 g (2 mmols) of H - Tyr - (i - BAC) - OCH_3.HBr and 0.95 g (2 mmols) of Z - Phe - OTCP were dissolved in 10 ml of dimethyl - formamide and, after cooling to —5°C, combined with 0.28 ml (2 mmols) of triethylamine. After having allowed the whole to stand for 60 hours at room temperature, it was evaporated under reduced pressure, the residue was dissolved in chloroform and worked up as described in Example 1c). Yield: 0.98 g (85% of the theory). Melting point: 196—198°C.

$C_{34}H_{37}N_3O_7$ (575.68) Calc.:
C=66.77 H=6.48 N=7.30
Found:
C=66.9 H=6.5 N=7.6

d) Z - Phe - Tyr - OH

0.58 g (1 mmol) of Z - Phe - Tyr - (i-BAC) - OCH_3 was dissolved in 7 ml of dimethyl - acetamide and 5 ml of dioxane and, after addition of 1.5 ml (3 mmols) of binormal sodium hydroxide solution, the whole was stirred for 2 hours at room temperature. The solution was diluted with 100 ml of water, combined with 1.75 ml of binormal hydrochloric acid, the precipitate that had separated was taken up in ethyl acetate and extracted with three portions of sodium hydrogenocarbonate solution. Upon addition of binormal hydrochloric acid, the crystalline Z-peptide precipitated which was washed with water and dried. Yield: 0.36 g (78% of the theory). Melting point: 187—188°C. A sample thereof was recrystallized for analysis from a mixture of ethyl acetate and petrol ether, whereupon the melting point was found to have risen to 189—189.5°C. Melting point in literature: 184—185°C [Liebigs Ann. Chem. 652, 79 (1962)].

$C_{26}H_{26}N_2O_6$ (462.51) Calc.:
C=67.52 H=5.67 N=6.06
Found:
C=67.7 H=5.8 N=6.0

Example 8

Z - Phe - Tyr - $NHNH_2$

1.15 g (2 mmols) of Z - Phe - Tyr - (i - BAC) - OCH_3 [prepared according to Example 7c)] were reacted as described in Example 6d) for 36 hours, at room temperature, in 8 ml of dimethyl - acetamide with 0.64 ml (10 mmols) of 80% hydrazine hydrate. Yield: 0.84 g (88% of the theory). Melting point: 224—225°C. The hydrazide showed the same properties as the product obtained according to Example 6d).

$C_{26}H_{29}N_4O_5$ (476.54) Calc.:
C=65.53 H=5.92 N=11.76
Found:
C=65.2 H=5.9 N=12.0

Example 9

a) Z - Tyr - (PAC) - OCH_3

3.29 g (10 mmols) of Z - Tyr - OCH_3 were dissolved in 20 ml of dimethylformamide. After cooling to 0°C, 1.31 g (11 mmols) of phenyl - isocyanate were added and the whole was allowed to stand for 50 hours. It was then evaporated in a high vacuum. The residue crystallized upon trituration with ligroin. The crystals were filtered off with suction and triturated with absolute ethanol, allowed to stand for 12 hours, filtered off with suction and washed with ethanol. Yield: 3.22 g (72% of the theory). Melting point: 140—141°C.

$C_{25}H_{24}N_2O_6$ (448.49) Calc.:
C=66.95 H=5.39 N=6.25
Found:
C=67.0 H=5.0 N=6.0

b) H - Tyr - (PAC) - OCH_3.HBr

4.48 g (10 mmols) of Z - Tyr - (PAC) - OCH_3 were reacted with 10 ml of a mixture of HBr and glacial acetic acid for one hour, at room temperature. After addition of 100 ml of absolute ether and short standing, the whole was suction-filtered and washed with ether. The crude product was triturated in hot ethyl acetate. Yield: 3.64 g (92% of the theory). Melting point: 205.5°C (decomposition).

$C_{17}H_{19}N_2O_4Br$ (395.26) Calc.:
C=51.66 H=4.85 N=7.09 Br=20.22
Found:
C=51.5 H=5.0 N=7.0 Br=20.4

c) Z - Phe - Tyr - (PAC) - OCH_3

α) 1.49 g (5 mmols) of Z - Phe - OH were dissolved in 20 ml of tetrahydrofurane. After the addition of 0.7 ml (5 mmols) of triethylamine, 0.48 ml (5 mmols) of chloroformic acid ethyl ester were added dropwise, at $-10°C$, while stirring. The precipitate that had separated was dissolved by the addition of 20 ml of dimethylformamide and 50 ml of chloroform. The whole was stirred for 5 minutes at $-5°C$ and then combined with the solution of 1.98 g (5 mmols) of H - Tyr - (PAC) - OCH_3.HBr and 0.7 ml of triethylamine in 40 ml of dimethylacetamide and 60 ml of chloroform, which had been cooled to $-5°C$. The temperature of the batch was allowed to rise slowly to room temperature and the mixture was stirred for one hour. After removal of the solvent by distillation under reduced pressure, the residue was taken up in 2.5 liters of chloroform and the solution was washed with sodium bicarbonate solution, 1N - hydrochloric acid and water, dried over sodium sulphate and evaporated under reduced pressure. The crude product (2.81 g=95% of the theory) melting at 182.5—185°C was recrystallized from a mixture of chloroform and petrol ether. Yield: 1.85 g (62% of the theory). Melting point 193—195°C.

$C_{31}H_{33}N_3O_7$ (595.67) Calc.:
C=68.56 H=5.58 N=7.05
Found:
C=67.9 H=5.5 N=6.8

β) 3.95 g (10 mmols) of H - Tyr - (PAC) - OCH_3.HBr and 4.77 g (10 mmols) of Z - Phe - OTCP were reacted in 30 ml of dimethylformamide according to Example 6c). Yield: 5.20 g (87% of the theory). Melting point: 195—196°C.

γ) Introduction of the PAC-protective group into Z - Phe - Tyr - OCH_3:

2.39 g (5 mmols) of Z - Phe - Tyr - OCH_3 [prepared according to the mixed anhydride method, melting point 143—144°C (melting point in literature: 137—138°C, Rec. Trav. Chim. Pays Bas 78, (1959), page 487)

$C_{27}H_{28}N_2O_6$ (476.54) Calc.:
C=68.05 H=5.93 N=5.88
Found:
C=68.3 H=6.1 N=5.8]

were dissolved in 20 ml of absolute dimethylformamide, the solution was cooled to $-5°C$ and combined with 0.66 g (5.5 mmols) of phenyl - isocyanate. After standing for 65 hours at room temperature, the solution was evaporated to dryness in a high vacuum, the solid residue was triturated with ligroin and filtered off with suction. The crude product was recrystallized twice from a mixture of chloroform, dimethyl - acetamide and petrol ether. Yield: 166 g (56% of the theory). Melting point 195—196.5°C.

d) Z - Phe - Tyr - OH

1.19 g (2 mmols) of Z - Phe - Tyr - (PAC) - OCH_3 were hydrolyzed in 14 ml of dimethyl - acetamide and 10 ml of dioxane with 3 ml (6 mmols) of binormal sodium hydroxide solution as described in Example 2d). Yield: 0.72 g (78% of the theory). Melting point: 185—187°C. Both Z - peptides were identical.

Example 10

Z - Phe - Tyr - $NHNH_2$

The reaction of 0.60 g (1 mmol) of Z - Phe - Tyr - (PAC) - OCH_3 [prepared according to Example 9c)] with 0.32 ml (5 mmols) of 80% hydrazine hydrate in 4 ml of dimethyl - acetamide was effected as described in Example 6d). The reaction time was 38 hours. Yield: 0.34 g (71% of the theory). Melting point: 224—224.5°C.

The two hydrazines had the same properties.

Example 11

a) BOC - Tyr - OBzl

60 g (0.22 mol) of H - Tyr - OBzl were stirred for 2 days, at room temperature, with BOC-azide in pyridine. Yield: 62.5 g (77% of the theory). Melting point: 126—127°C.

$C_{21}H_{25}NO_5$ (371.24) Calc.:
C=67.94 H=6.79 N=3.77
Found:
C=68.0 H=6.8 N=3.7

b) BOC - Tyr - (PAC) - OBzl

7.42 g (20 mmols) of BOC - Tyr - OBzl were dissolved in 50 ml of absolute dimethylformamide and, after cooling to 0°C, combined with 2.62 g (22 mols) of phenyl - iso - cyanate. After standing for 50 hours at room temperature, the solvent was removed by distillation in a high vacuum and the oily residue was triturated twice with ligroin, whereby partial crystallization took place. Upon dissolution in cold methanol and precipitation with water, the product crystallized thoroughly and was then recrystallized from a mixture of hot ethanol and water. Yield: 7.10 g (72% of the theory). Melting point: 108—108.5° C.

$C_{29}H_{30}N_2O_6$ (490.57) Calc.:
C=68.55 H=6.16 N=5.71
Found:
C=68.5 H=6.3 N=5.8

c) BOC - Tyr - (PAC) - OH

3.8 g (7.75 mmols) of BOC - Tyr - (PAC) - OBzl were dissolved in 120 ml of methanol and hydrogenated for 30 minutes in the presence of palladium black. The

catalyst was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and the product was precipitated by adding petrol ether. Yield: 2.6 g (84% of the theory). Melting point 125—130°C.

$C_{21}H_{24}N_2O_6$ (400.44) Calc.:
C=62.99 H=6.04 N=7.00
Found:
C=63.1 H=6.2 N=7.2

d) BOC - Tyr - (PAC) - OTCP

2.00 g (5 mmols) of BOC - Tyr - (PAC)-OH and 1.19 g (6 mmols) of 2,4,5 - tri - chlorophenol were dissolved in 45 ml of ethyl acetate and combined, at 0°C, with 1.05 g (5.1 mmols) of dicyclohexyl - carbodiimide. After having allowed the whole to stand for 16 hours at 5°C, the urea was removed by filtration with suction, the filtrate was evaporated to dryness under reduced pressure and the crystalline residue was recrystallized from isopropanol. Yield: 0.94 g (32% of the theory). Melting point 162°C.

$C_{27}H_{25}N_2O_6Cl_3$ (579.89) Calc.:
C 55.93 H 4.35 N 4.83 Cl 18.34
Found:
C 56.0 H 4.5 N 4.8 Cl 18.1

e) BOC - Tyr - (PAC) - Phe - OCH_3

0.58 g (1 mmol) of BOC - Tyr - (PAC) - OTCP and 0.22 g (1 mmol) of H - Phe - OCH_3.HCl were dissolved in 3 ml of dimethylformamide and combined, at −5°C, with 0.14 ml (1 mmol) of triethylamine. After having allowed the whole to stand for 30 hours at room temperature, it was evaporated under reduced pressure, the semi-solid residue was dissolved in a mixture of chloroform and ethyl acetate and washed, dried and evaporated as described in Example 6c). The residue was triturated with a mixture of ether and petrol ether, filtered off with suction and washed with petrol ether. Yield: 0.39 g (70% of the theory). Melting point: 152—153°C (sintering from 115°C).

$C_{31}H_{35}N_3O_7$ (561.64) Calc.:
C=66.30 H=6.28 N=7.48
Found:
C=66.5 H=6.3 N=7.5

f) BOC - Tyr - Phe - $NHNH_2$

BOC - Tyr - (PAC) - Phe - OCH_3 was subjected to hydrazinolysis as described in Example 6d). Yield: 60% of the theory. Melting point: 208°C.

EXAMPLE 12

Introduction and separation of the NPAC-group

a) Z - Tyr - (NPAC) - OCH_3

6.6 g (20 mmols) of Z - Tyr - OCH_3 were dissolved in 25 ml of dimethylformamide and cooled to −5°C. To this solution, there was added a solution of 3.6 g (22 mmols) of p - nitrophenyl - isocyanate in 60 ml of dimethylformamide, which had been cooled to −5°C, and the total solution was allowed to stand for 24 hours at room temperature. The urea that had precipitated was filtered off with suction and the filtrate was evaporated in a high vacuum. The residue was triturated twice with ligroin and decanted. The crude product was dissolved in acetone, the resulting solution was poured onto a column filled with neutral aluminium oxide and elution was effected with a mitxure of n - hexane, ethyl acetate and glacial acetic acid (20:10:1). The solution containing the first appearing substance was evaporated under reduced pressure and the solid residue was recrystallized from hot methanol. Yield: 3.22 g (33% of the theory). Melting point: 179—180°S.

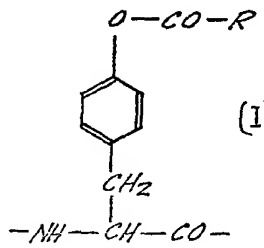
$C_{25}H_{23}N_3O_8$ (493.49) Calc.:
C=60.85 H=4.70 N=8.52
Found:
C=60.6 H=4.7 N=8.6

b) Z - Tyr - $NHNH_2$

0.49 g (1 mmol) of Z - Tyr - (NPAC) - OCH_3 was dissolved in 3 ml of dimethyl - acetamide and allowed to react for 22 hours, at room temperature, with 0.32 ml (5 mmols) of 80% hydrazine hydrate. After evaporation, the residue was triturated with methanol and filtered off with suction. The crude hydrazide was recrystallized from methanol. Yield: 0.21 g (64% of the theory). Melting point: 220.5—221°C (decomposition). The substance was identical with Z - Tyr - $NHNH_2$.

WHAT WE CLAIM IS:—

1. A process for the manufacture of tyrosine-containing peptides, wherein a peptide containing at least one tyrosine unit of the general formula



(I)

in which R represents an alkoxy radical derived from a primary or secondary alcohol, an aralkoxy radical containing at least 2 carbon atoms between the phenyl nucleus and the oxygen atom or an NHR_1- group in which R_1 represents a hydrogen atom or an alkyl, aralkyl or aryl radical, is

a) treated with ammonia, an amine, a hydrazine or, when R is not an NHR_1 group, with a mono - acyl - hydrazine, the amine or hydrazine derivative containing at least one NH-group; or

b) subjected to alkaline hydrolysis; or

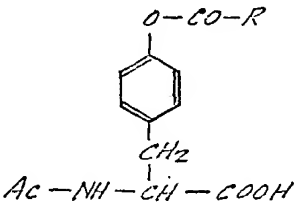
c) treated with an alkali metal alcoholate; or

d) treated with a solution of an alkali or alkaline earth metal in liquid ammonia.

2. A process as claimed in claim 1, conducted substantially as described in any one of the Examples herein.

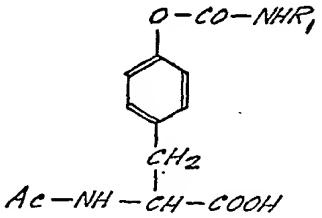
3. Tyrosine-containing peptides whenever obtained by the process claimed in claim 1 or claim 2.

4. Tyrosine derivatives of the general formula



wherein Ac represents a carbobenzoxy, tertiary butyloxy - carbonyl or a formyl radical and R represents an alkoxy radical containing from 1 to 4 carbon atoms.

5. Tyrosine derivatives of the general formula



wherein Ac represents a carbobenzoxy or a tertiary butyloxy - carbonyl radical and R_1 represents a hydrogen atom or an isobutyl, phenyl or 4 - nitrophenyl radical.

6. Any one of the tyrosine-containing peptides obtainable by the process of claim 1 and described in the Examples herein.

7. A pharmaceutical preparation which comprises a compound as claimed in claim 6 in admixture or conjunction with a pharmaceutically suitable carrier.

8. A peptide containing at least one tyrosine unit of the general formula I as defined in claim 1.

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